

CLAIMS

1. A sorbitol dehydrogenase having the following physicochemical properties:

- (a) action: catalyzes the reaction converting D-sorbitol to
5 L-sorbose
- (b) molecular weight: about 54 kDa
- (c) coenzyme: NAD(P)⁺ dependent
- (d) substrate specificity: specifically oxidizes sorbitol, mannitol and arabitol, but does not act on xylitol, ribitol,
10 inositol or glycerol.

2. The sorbitol dehydrogenase of claim 1, which is derived from the strain *Gluconobacter oxydans* G624.

- 15 3. A sorbitol dehydrogenase which is originated from the same gene as is the sorbitol dehydrogenase of claim 2 in its molecular evolution.

- 20 4. The sorbitol dehydrogenase of claim 3, which is derived from a bacteria belonging to the genus *Gluconobacter*.

5. A sorbitol dehydrogenase which is the following protein

(a) or (b):

- (a) a protein consisting of an amino acid sequence depicted
25 in Sequence Listing SEQ ID NO:1

- (b) a protein consisting of the same amino acid sequence as

(a) above, except that one to several amino acids is(are) deleted, substituted, inserted, added or modified, and catalyzing the reaction converting D-sorbitol to L-sorbose.

5 6. A DNA encoding the sorbitol dehydrogenase of any of claims 1 to 5.

7. The DNA of claim 6, which is (a) or (b) in the following:

(a) a DNA having a base sequence of base numbers 537 - 1991
10 of the base sequence depicted in Sequence Listing SEQ ID NO:2

(b) a DNA capable of hybridizing to the base sequence of the above-mentioned (a) under stringent conditions.

8. The DNA of claim 6 or 7, which is derived from bacteria
15 belonging to the genus *Gluconobacter*.

9. A gene encoding a protein having a sorbitol dehydrogenase activity, which is a DNA capable of hybridizing a DNA having a base sequence of base numbers 537 - 1991 of the base
20 sequence depicted in Sequence Listing SEQ ID NO:2 and a partial DNA thereof.

10. A protein derived from the genus *Gluconobacter*, which is encoded by the gene of claim 9 and which has a sorbitol
25 dehydrogenase activity.

11. A promoter gene comprising the DNA of the following (a)
or (b):

(a) a DNA having a base sequence of base numbers 1 - 536 of
the base sequence depicted in Sequence Listing SEQ ID NO:2

5 (b) a DNA having a base sequence of the above-mentioned (a)
wherein one to several bases is(are) deleted, substituted,
inserted, added or modified, which DNA shows a promoter
activity at least in one kind of microorganism.

10 12. A recombinant vector comprising a DNA of any of claims 6
to 9.

Sub-G15 13. An expression vector comprising a DNA of any of claims 6
to 9.

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14. The expression vector of claim 13 further comprising a
DNA encoding sorbose dehydrogenase and/or a DNA encoding
sorbose dehydrogenase.

Sub-G16 20 15. A transformant obtained by transforming a host cell with
an expression vector of claim 13 or 14.

16. The transformant of claim 15, which belongs to a genus
selected from the group consisting of *Escherichia coli*, the
25 genus *Pseudomonas*, the genus *Gluconobacter*, the genus
Acetobacter and the genus *Pseudogluconobacter*.

17. The transformant of claim 15 or 16, which is capable of converting D-sorbitol to 2-keto-L-gulonic acid.

5 18. A method for producing a protein having a sorbitol dehydrogenase activity, which method comprises culturing a host cell transformed with an expression vector of claim 13 in a medium and harvesting the sorbitol dehydrogenase of any of claims 1 to 5 or the protein of claim 10 from the obtained
10 culture.

19. A method for producing an L-sorbose, which method comprises culturing a host cell transformed with an expression vector of claim 13 in a medium and bringing D-
15 sorbitol into contact with the obtained culture or a treated product thereof.

20. A method for producing 2-keto-L-gulonic acid, which method comprises culturing a host cell transformed with an
20 expression vector containing a DNA encoding sorbose dehydrogenase and a DNA encoding sorbosone dehydrogenase in a medium and bringing the L-sorbose obtained according to the method of claim 19 into contact with the obtained culture or a treated product thereof.

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21. A method for producing 2-keto-L-gulonic acid, which

method comprises culturing the transformant of claim 17 in a medium and bringing D-sorbitol into contact with the obtained culture or a treated product thereof.

5 22. A method for producing L-ascorbic acid or an alkali metal salt thereof or an alkaline earth metal salt thereof, which method comprises converting 2-keto-L-gulonic acid obtained by the method of claim 20 or 21 to L-ascorbic acid or an alkali metal salt thereof or an alkaline earth metal salt thereof.

Sub a18

Add a19